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User manual

imegen® SCAs kit

SCA1, SCA2, SCA3, SCA6 and SCA7
expansions detection by PCR and TP-PCR

Reference: IMG-152

Made in Spain

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All products sold by the Imegen are subjected to rigorous quality control. The **imegen® SCAs kit** has passed all internal validation tests, ensuring the reliability and reproducibility of each assay.

For any questions about the applications of this product or its protocols, please contact our Technical Department:

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1 Kit description

Product description

Spinocerebellar ataxias (SCAs) comprise a heterogeneous group of neurodegenerative diseases characterized primarily by progressive gait disturbance and to present a pattern of autosomal dominant inheritance. Different genes have been described in this pathology which mutation usually consists in the CAG repetition expansion.

Imegen®SCAs kit allows the CAG expansion analysis of the SCA1, SCA2, SCA3, SCA6 and SCA7 by PCR and TP-PCR.

	Gen	Chromosome location	Repetitions Number (CAG)n*			
			Normal Allele	Uncertain Allele	Reduced penentrance Allele	Expanded Allele. Pathological
SCA1 (MIM#164400)	ATXN1	6p22.3	6-39	-	-	40-82
SCA2 (MIM#183090)	ATXN2	12q24.13	14-31	32-34	-	35-500
SCA3 (MIM#109150)	ATXN3	14q32.12	11-44	45-59	-	61-87
SCA6 (MIM#183086)	CACNA1A	19p13.13	4-18	-	19	20-33
SCA7 (MIM#164500)	ATXN7	3p14.1	4-19	28-33	34-35	36-460

*Table 1. Information about SCAs included in **imegen®-SCAs Kit***

Content and storage of the kit

The kit contents the necessary reagents to perform 12 samples:

Reagents	Identification	Amount	Storage
General-AD Master Mix	White pad	900 µl	-20ºC
General-EG Master Mix	Yellow pad	685 µl	-20ºC
AD Taq	Orange cap	30 µl	-20ºC
EG Taq	Yellow cap	15 µl	-20ºC
Specific SCA Master Mixes Strip	-	7 x 66 µl	-20ºC

*Table 2. Kit components and storage temperature of **imegen®-SCAs kit***

NOTE: Reagents required but not provided: reagents for capillary electrophoresis and consumables.

Each SCA-specific master mix is included in the 8-wells strip, provided by this kit, with the following distribution:

Well	SCA Master Mix	Analysis Type
A	SCA1	PCR
B	SCA2	PCR
C	SCA3	PCR
D	SCA6	PCR
E	SCA7	PCR
F	SCA2	TP-PCR
G	SCA7	TP-PCR

Table 3. *8-wells strip distribution*

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2 Amplification by PCR and TP-PCR

Equipment and material required

In the following table the equipment requirements for using **Imegen-SCAs Kit** are shown:

Equipment	
1	PCR Thermal Cycler
2	Multichannel pipette (0.5 – 10 µL)
3	Micropipettes (10 µL, 20 µL and 200 µL)
4	Vortex

***Table 4**. Equipment requirements*

Needed consumables are shown in the following table:

Materials	
1	Disposable micropipette filter tips (10 µL, 20 µL and 200 µL)
2	96-well plates or 0,2 mL tubes
3	1.5 mL sterile tubes
4	Powder-free latex gloves

***Table 5**. Nedeed materials*

PCR reactions preparation

Imegen-SCAs Kit is designed to perform 7 reactions per sample (5 PCRs and 2 TP-PCRs).

To estimate the amount of necessary reagents, we recommend make calculations taking into account the number of samples to be simultaneously analysed, and then considering one more reaction, or increase a 10% the volume of each reagent.

To perform the analyses of expansions analysed using **imegen®-SCAs kit**, two master mixes should be prepared: one for PCR reactions (SCA1, SCA2, SCA3 and SCA6) and the other for TP-PCR reactions (SCA2 and SCA7).

Recommended protocol for preparation of amplification reactions is showed below:

1. Thaw all reagents contained in the kit and samples DNA.
2. Vortex each reagent and keep cold.
3. In order to prepare the PCR master mix, add into a 1.5 mL tube, the following reagents:

Reagent	Amount per sample or control
General-AD Master Mix	68 µL
AD Taq	2 µL

Table 6. *PCR Master Mix reagents*

4. In order to prepare the TP-PCR master mix, add into a 1.5 mL tube, the following reagents:

Reagent	Amount per sample or control
General-EG Master Mix	52 µL
EG Taq	2 µL

Table 7. *TP-PCR Master Mix reagents*

5. Vortex and spin PCR and TP-PCR Master Mix tubes and dispense 17.5 µL of the PCR Master Mix in corresponding wells of A, B, C and D rows of the plate. It should be filled many columns as samples or controls are going to be simultaneously analysed.
6. Dispense 17.5 µL of the TP-PCR Master Mix in corresponding wells of E,F and G rows of the plate. It should be filled many columns as samples or controls are going to be simultaneously analysed.
7. Add 5 µL of the SCA-specific master mixes included in the kit' s strip with a multichannel pipette to each column depending on the samples or controls.
8. Add 2.5 µL of sample DNA at 10 ng/µL into each reaction well. It is recommended to add a PCR negative control in order to rule out PCR contamination, and also positive controls to verify the allele' s sizes.

PCR amplification program

Amplification reactions should be summited to the following PCR program:

- Optimal program:

Fields	Step 1 Enzyme activation	Step 2 PCR			Step3 Extension	Step4 Conservation
Cycles	1 initial cycle	30 cycles			1 cycle	∞
		Denaturalization	Primers binding	Extension		
Temperature	94ºC	94ºC	60ºC	72ºC	72ºC	4ºC
Time	5 minutes	1 minute	1 minute	2 minutes	10 minutes	1 minute

Table 8. *Optimal program for T3 (Biometra) or GENEAMP® PCR system 2720 (Applied Biosystems) thermal cycler.*
*

Note: This program has been validated on Biometra T3 equipment and GeneAmp® PCR System 2720 (Applied Biosystems). If another thermocycler is going to be used, maybe, it would be necessary to adjust the amplification program. Please contact our customer service if you need advice.

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Fragment analysis

Fragment analysis preparation

In order to perform the expansions analysis, a fragment analysis is required. Thus, a fragments plate with PCR and TP-PCR products has to be prepared. Recommended protocol for its preparation is showed below:

1. Add into a 1.5 mL tube, the following reagents:

Reagent	Amount per obtained reactions
Formamide	18 µL
GeneScan™ 500 LIZ marker	0.5 µL

Table 9. *Fragment plate Master Mix reagents*

Note: To estimate the amount of necessary reagents, we recommend make calculations taking into account the number of samples to be simultaneously analysed, and then considering one more reaction, or increase a 10% the volume of each reagent.

2. Dispense 18.5 µL of the Fragment plate Master Mix in each well.
3. Add 1 µL of the DNA obtained in PCR and TP-PCR reactions.
4. Cover and spin the plate and denature in a thermal cycler for 5 minutes at 98ºC.
5. Store the plate at 4ºC until the moment of the capillary electrophoresis.

Capillary electrophoresis

Once prepared fragments plate, reactions should be subjected to capillary electrophoresis. Depending on the type of sequencer used, electrophoresis conditions recommended by the manufacturer shall be used. To set these conditions, it should be taken into account that amplification range varies approximately between 100-500 pb, that are employed FAM-labelled primers and that molecular weight standard is labelled with LIZ.

Detection intensity may vary between different equipment, depending on the model and the conditions of the equipment optical system. Therefore in some cases it may be necessary to dilute the samples before performing capillary electrophoresis.

4 Analysis results

Between different laboratories it has been described variation in one repetition, depending on reagents and capillary electrophoresis equipment used for fragment analysis. To calculate the exact number of repetitions of an unknown allele can use the following formula:

$$Repetitions\ Number = \frac{Size_{Allele\ x} - X\ pb}{3}$$

where **X** is different from each SCA:

SCA	X pb
SCA1	142
SCA2	103
SCA3	160
SCA6	102
SCA7	263

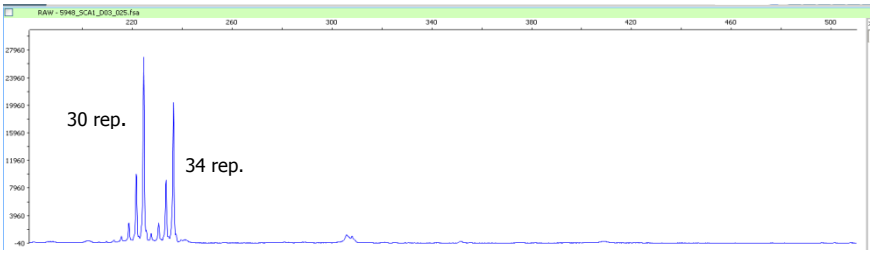
Table 10. *SCAs normal allele size*

Or use a sample with a known repetition size (for example: 8 repetitions):

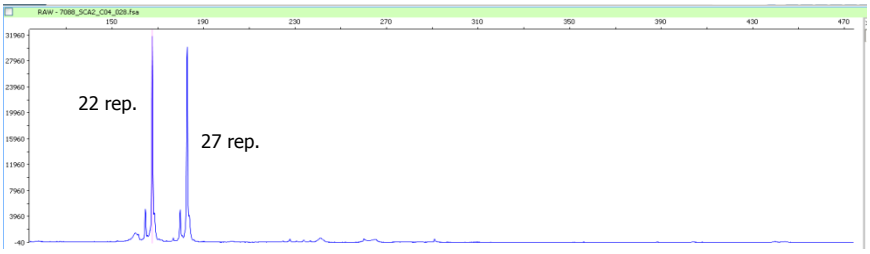
$$Repetitions\ Number = \frac{Size_{Allele\ x} - Size_{Allele\ 8\ rep.}}{3} + 8$$

PCR results:

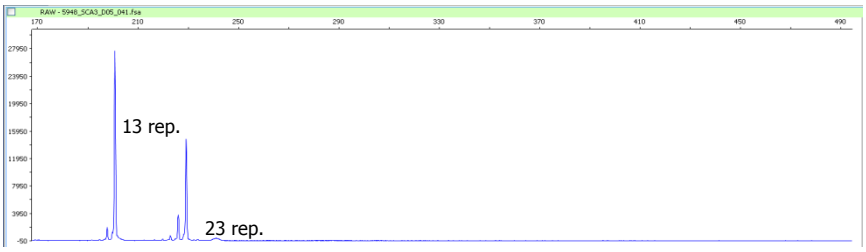
SCA1



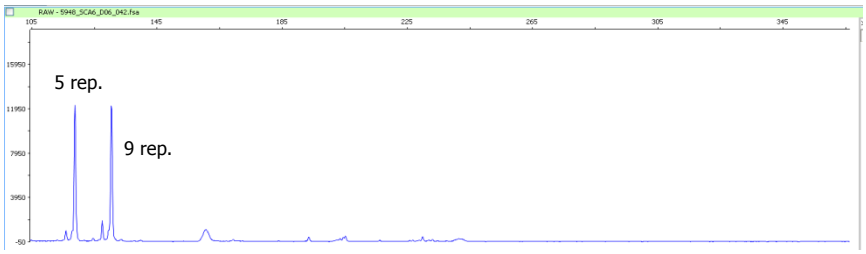
SCA2



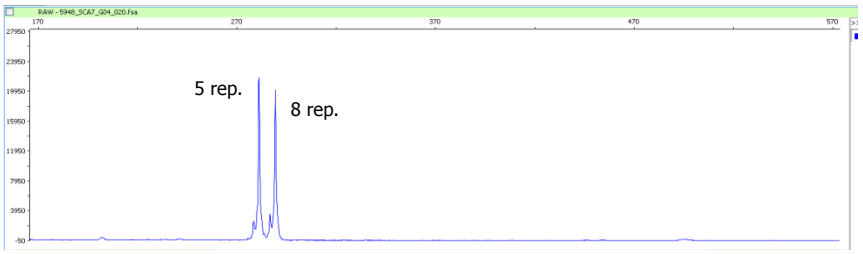
SCA3



SCA6



SCA7

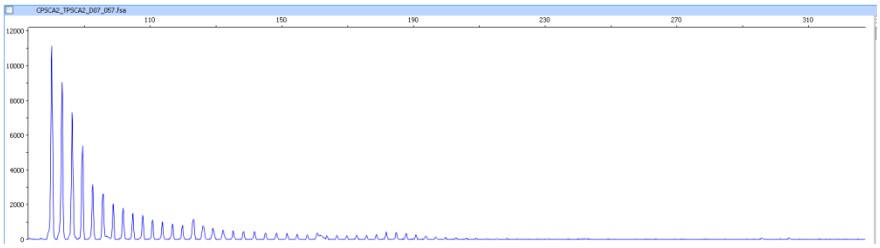


TP-PCR results:

It is necessary to evaluate the TP-PCR results (Triplet Repeat primed polymerase chain reaction) in those samples where only one allele is detected by using PCR. TP-PCR differentiates between normal allele homozygous samples and expanded alleles with samples that cannot be amplified by conventional PCR.

This technique allows the detection of expansions of any size, although smaller allele identification could be complicated and generally does not allow determining the number of repetitions.

SCA2



SCA7